UNITED STATES ENVIRONMENTAL PROTECTION
AGENCY
WASHINGTON, D.C. 20460

EPA United States Environmental Protection Agency **Office of Pesticide Programs**

Thursday, April 09, 2015

MEMORANDUM

Subject: Acute Toxicity Review for EPA Reg. No.: 1677-ELN
DP Barcode: D424060
Product Name: Synergex

From: Ian Blackwell, Biologist
Chemistry and Toxicology Team
Product Science Branch
Antimicrobials Division (7510P)

Through: for Karen Hicks, Team Leader
Chemistry and Toxicology Team (CTT)
Product Science Branch
Antimicrobials Division (7510P)

To: Elizabeth Watkins, Acting PM 33
Regulatory Management Branch
Antimicrobials Division (7510P)

Applicant: EcoLab, Inc.

FORMULATION FROM LABEL:

PC Code	Active Ingredient(s):	% by wt.
000595	Hydrogen Peroxide	10.70
063209	Peroxyoctanoic Acid	0.63
063201	Peroxyacetic Acid	2.38
	Other Ingredient(s):	86.29
	Total:	100.00

I BACKGROUND: EcoLab, Inc. has submitted a complete set of six acute toxicity studies in support of their product, "Synergex". MB Research Laboratories conducted these studies. The test material is identified as "KX-6228". However, the registrant's 10/20/2014 cover letter states that this refers to the product, Synergex. In addition to testing of the undiluted registration product, the registrant also had the lab test of 2% solutions of the product.

EcoLab asks for waivers of the primary eye and skin irritation studies for the concentrate (undiluted version) of this product. The basis of the waivers is that the pH of Synergex is 1.6. This falls within the area of waivers for such studies suggested by the Agency.

This submission includes a dermal sensitization study conducted on the concentrate using the Local Lymph Node Assay, LLNA-BrdU ELISA method. This method is new to the Antimicrobials Division. There was no dermal sensitization study submitted for the 2% use-solution.

II RECOMMENDATIONS:

1. The acute oral and inhalation toxicity studies are acceptable.

2. CTT must assign toxicity category I to Synergex (1677-ELN) for acute dermal toxicity. This product delivered extreme results in that it, at the Limit Test dosage of 2,000 mg/kg, it caused convulsions, coma and death **within ten minutes** in two-out-of-two of the test subjects. Also, this product was generally so toxic that the test facility chose not to test the standard five male and five female animals at each dose.

   The results of this acute dermal toxicity study might place this product into Toxicity Category II. The corresponding precautionary labeling for this product would state "May be fatal if absorbed through skin." The Chemistry and Toxicology Team (CTT) along with AD toxicologists feel that there is a strong possibility this statement underestimates the hazards of File Symbol 1677-ELN.

3. CTT waives the primary eye irritation study for 1677-ELN concentrate. The pH of the product is reported to be 1.5 – 1.7 on the CSF for a 1% solution. Also, CTT found the product to be corrosive and toxic in the acute dermal toxicity study. CTT assigns a corresponding toxicity category I for primary eye irritation.

4. CTT waives the primary skin irritation study for 1677-ELN concentrate). The pH of the product is reported to be 1.5 – 1.7 on the CSF for a 1% solution. Also, the laboratory found the product to be corrosive and toxic in the acute dermal toxicity study. CTT assigns a corresponding toxicity category I for primary skin irritation.

5. CTT must reject the dermal sensitization study, MRID Number 49467424. The problem is that, at this time, the Office of Pesticide Programs (OPP) does not accept the Local Lymph Node Assay conducted using the LLNA-BrdU ELISA method.

The acute toxicity profile for File Symbol 1677-ELN is currently:

Acute Toxicity Testing of Undiluted Synergex			
Study	MRID Number	Toxicity Category	Study Status
Acute Oral Toxicity	49467521	III	Acceptable
Acute Dermal Toxicity	49467422	I	Acceptable
Acute Inhalation Toxicity	49467523	IV	Acceptable
Primary Eye Irritation	49467430	I	Waived
Primary Skin Irritation	49468731	I	Waived
Dermal Sensitization	49467424	---	Unacceptable

Acute Toxicity Profile of 2% Synergex			
Study	MRID Number	Toxicity Category	Study Status
Acute Oral Toxicity	49467425	IV	Acceptable
Acute Dermal Toxicity	49467426	IV	Acceptable
Acute Inhalation Toxicity	49467427	IV	Acceptable
Primary Eye Irritation	49467429	III	Acceptable
Primary Skin Irritation	49467428	IV	Acceptable
Dermal Sensitization	None	---	--

III LABELING:

1. The Signal Word is DANGER based upon the classification of the primary eye and skin irritation and acute dermal toxicity studies.

2. CTT cannot prescribe further precautionary labeling due to the lack of an acceptable dermal sensitization study.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (§ 81-1, 870.1100)

Product Manager: 33 **Reviewer:** Ian Blackwell
MRID No.: 49467521 **Study Completion Date:** 9/9/2014
Lab Study No.: MB 14-22489.01

Testing Laboratory: MB Research Laboratories
Authors: Blair Yasso, B.S.

Quality Assurance (40 CFR §160.12): Included

Test Material: KX-6228 Concentrate; Clear colorless liquid

Species: Sprague Dawley rats
Weight: 192 - 246 grams **Age:** 10 weeks
Source: Charles River

Conclusion:

1. LD_{50} (mg/kg): **Males=** 2,000
Females= 2,000
Combined= 2,000

2. The estimated LD_{50} is 2,000 mg/kg of body weight.
3. Tox. Category: III **Classification:** Acceptable

Procedure (Deviations from §81-1): None

Method: Up and Down Procedure

Results:

Dosage (mg/kg)	(Number Deaths/Number Tested)		
	Males	Females	Combined
175	---	0/1	---
550	---	0/4	---
2000	---	4/5	---

Observations: Wet and yellow anogenital area; nose/muzzle red and wet; brown staining of head, wetness and brown staining of abdomen; chromodacryorrhea, piloerection, sagging eyelids, lethargy, hunched posture, prostration; cold to touch, ataxia, sagging lower lip; lack of feces, diarrhea; emaciation; bloated abdomen; flaccid muscle tone; negative righting reflex, tachypnea (rapid breathing).

Gross Necropsy: Anogenital area and abdomen having wetness and yellow/brown staining; excess fluid in the pleural and peritoneal cavity; a 2 mm ulceration on stomach; dark red and pale areas in intestines, distended intestines; chromodacryorrhea, chromorhinorrhea; red staining of muzzle; pale kidneys; liver had adhesion to the outer wall of the stomach; rupturing of the stomach.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (§ 81-1, 870.1100)

Product Manager: 33 **Reviewer:** Ian Blackwell
MRID No.: 49467425 **Study Completion Date:** 6/24/2014
MB Research Project No.: MB 14-22490.1

Testing Laboratory: MB Research Laboratories
Authors: Blair Yasso, B.S.

Quality Assurance (40 CFR §160.12): Included

Test Material: KX-6228, 2% (v/v) use-solution; Clear colorless liquid

Species: Sprague-Dawley rats
Weight: 206-228 g **Age:** Approx. 14 weeks
Source: Charles River

Conclusion:

1. **LD_{50} (mg/kg):** **Males=** Not tested
Females= > 5,000 mg/kg
Combined= Not tested
2. **The estimated LD_{50} is greater than 5,000 mg/kg of body weight.**

3. **Tox. Category:** IV **Classification:** Acceptable

Procedure (Deviations from §81-1): None

Method: Up and Down Procedure

Results:

Dosage (mg/kg)	(Number Deaths/Number Tested)		
	Males	Females	Combined
5, 000 mg/kg	---	0/3	---

Observations: Hair loss, eschar, wet anogenital area.

Gross Necropsy: Eschar on ear, hair loss on lower abdomen.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (§81-2, 870.1200)

Product Manager: 33 **Reviewer:** I. Blackwell
MRID No.: 49467422 **Study Completion Date:** 9/17/2014
MB Research Project No.: MB 14-22489.02

Testing Laboratory: MB Research Laboratories
Author: Blair Yasso, B.S.
Quality Assurance (40 CFR §160.12): Included

Test Material: KX-6228 Concentrate; Clear colorless liquid

Species: New Zealand White rabbits
Weight: Males= 2.6 – 3.0 kg **Age:** 23 weeks
Females= 2.8 – 3.1 kg
Source: Covance Research Products, Inc.,

Summary:

1. **LD_{50} (mg/kg):**
   Males= 300 < X < 750 mg/kg b.w.
   Females= 300 < X < 750 mg/kg b.w.
   Combined= 300 < X < 750 mg/kg b.w.

2. **The estimated LD_{50} is greater than 300; but, less than 750 mg/kg.**

3. **Tox. Category:** I **Classification:** Acceptable

Procedure (Deviation From §81-2): Due to the extreme effects of the product, the lab did not test 5 + 5 animals per dosage. Two dosages were miscalculated (1160 + 1167).

Results:

Reported Mortality

Dosage (mg/kg)	(Number Deaths/Number Tested)		
	Males	Females	Combined
300	1/5	1/5	2/10
750	2/2	3/3	5/5
1160	1/1	---	1/1
1167	1/1	---	1/1
2,000	2/2	---	2/2

Dose vs. Time to Mortality	
Dosage (mg/kg)	Time to Mortality
300	8/10 survived treatment
750	≤ 30 minutes
1,160-1,167	≤ 40 minutes
2,000	≤ 10 minutes

Observations: Erythema, edema, pale areas of skin, shiny areas, dark areas, cracked skin, **necrosis**, hyperactivity, convulsions, ataxia, coma, death, labored breathing, vocalizations. The two males tested at 2,000 mg/kg died within ten minutes of dosing.
Gross Necropsy Findings: Dose site pale. Otherwise appeared normal.

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (§81-2, 870.1200)

Product Manager: 33 **Reviewer:** I. Blackwell
MRID No.: 49467426 **Study Completion Date:** 6/24/2014
MB Research Project No.: MB 14-22490.02
Testing Laboratory: MB Research Laboratories
Author: Blair Yasso, B.S.
Quality Assurance (40 CFR §160.12): Included

Test Material: KX-6228, 2% (v/v) use-solution; Clear colorless liquid

Species: New Zealand White rabbits
Weight: Males= 2.6 – 2.9 kg **Age:** Approx. 22 weeks
Females= 2.8 – 3.1 kg
Source: Covance Research Products, Inc.

Summary:

1. **LD_{50} (mg/kg):**

Males	> 5,000
Females	> 5,000
Combined	> 5,000

2. **The estimated LD_{50} is greater than 5,000 mg/kg of body weight.**

3. **Tox. Category:** IV **Classification:** Acceptable

Procedure (Deviation From §81-2): None

Results:

Reported Mortality

DOSAGE (mg/kg)	(NUMBER DEATHS/NUMBER TESTED)		
	Males	**Females**	**Combined**
5,000	0/5	0/5	0/10

Observations: No abnormalities.

Gross Necropsy Findings: The lab found no abnormalities.

DATA REVIEW FOR ACUTE INHALATION TOXICITY (§81-3, 870.1300)

Product Manager: 33 **Reviewer:** I. Blackwell
MRID No.: 49467423 **Study Completion Date:** 9/25/2014
MB Research Project No.: MB 14-22489.05

Testing Laboratory: MB Research Laboratories
Author: Blair Yasso, B.S.

Quality Assurance (40 CFR §160.12): Included

Test Material: KX-6228 Concentrate; Clear colorless liquid, Lot P120331
Concentration: 0.53, 2.16 mg/L

Species: Sprague Dawley rats
Weight: Males= 323-461 g Females= 198-254 g
Age: approx. 14 weeks
Source: Charles River

Summary:

1. **LC_{50} (mg/L)** **Males** > 2.16 mg/L
   Females > 2.16 mg/L
   Combined > 2.16 mg/L
2. **The estimated LC_{50} is greater than 2.16 mg/L mg/L of air.**
3. **MMAD:** See Table below **µm**
4. **Toxicity Category:** IV **Classification:** Acceptable

Procedure (Deviation From §81-3): None

Results:

Reported Mortality

Exposure Concentration	(NUMBER DEATHS/NUMBER TESTED)		
	Males	Females	Combined
0.53 mg/L	0/5	0/5	0/10
2.16 mg/L	0/5	1/5	1/10

Chamber Atmosphere		
Dose Level	MMAD	GSD
0.53 mg/L	1.81 μm	1.99 μm
2.16 mg/L	1.74 μm	2.01 μm

Chamber Environment		
	0.53 mg/L	2.16 mg/L
Chamber Volume	100 liter	100 liter
Airflow	28.3 LPM	28.3 LPM
Temperature	21-22° C	21-23° C
Relative Humidity	72%	72%

Clinical Observations: The lab reported closed eyes, tears, eyes crusting, wetness of the nose/mouth area, test substance coated fur, irregular breathing, piloerection, wetness of the anogenital area, licking of the inside of the mouth, moderate erythema on extremities, lethargy, open mouth breathing, and hunched position. In the surviving animals, the lab reported unkempt appearance, opened-mouth and irregular breathing. On the skin, there was moderate erythema on extremities, slight to moderate edema on extremities, brown stained fur, pale areas on extremities, nose/mouth area pale, nose/mouth area crusted and stained brown and black, ocular opacities, hair foss on the nose, eye areas, abdomen, and lower back, nose/mouth area stained red, wetness and yellow staining of the anogenital area, closed eye {right), brawn staining of the fur on the head and face, flaking skin or eschar on face, nose and extremities, dyspnea, chromodacryorrhea, few feces, lethargy, chromorhinorrhea, and test article coated fur, were observed.

Gross Necropsy Findings: Eschar formation around nose, tail, above eyes and on the ears, extremities and face. Unkempt appearance, ocular opacity. brown-stained fur; yellow-stained anogenital area; flaking skin, erythema and edema on extremities, pale areas on extremities, nose and mouth. Test material covering fur; eschar on front limbs. Chromodacryorrhea, chromorhinorrhea. Hair loss on the nose/mouth area and eye area, forearms, chest, lower abdomen, lower back, and neck. Body weight loss was observed at death. The necropsy revealed ocular opacity. There were pale areas on the liver, distention of the stomach with gas and thick dark fluid, and distention of the intestines with thick, dark fluid.

DATA REVIEW FOR ACUTE INHALATION TOXICITY (§81-3, 870.1300)

Product Manager: 33 **Reviewer:** I. Blackwell
MRID No.: 49467427 **Study Completion Date:** 9/25/2014
MB Research Project No.: MB 14-22490.05

Testing Laboratory: MB Research Laboratories
Author: Blair Yasso, B.S.

Quality Assurance (40 CFR §160.12): Included

Test Material: KX-6228, 2% (v/v) use-solution; Clear colorless liquid
Concentration: 2.07 mg/L

Species: Sprague Dawley rats
Weight: Males= 261 – 377 g Females= 228 – 259 g
Age:
Source: Charles River

Summary:

1. **LC_{50} (mg/L)** **Males=** > 2.07 mg/L of air
   Females= > 2.07 mg/L of air
   Combined= > 2.07 mg/L of air
2. **The estimated LC_{50} is greater than 2.07 mg/L.**
3. **MMAD:** 2.06 µm **µm**
4. **Toxicity Category:** IV **Classification:** Acceptable

Procedure (Deviation From §81-3):

Results:

Reported Mortality

Exposure Concentration	(NUMBER DEATHS/NUMBER TESTED)		
	Males	Females	Combined
2.07 mg/L	0/5	0/5	0/10

Chamber Atmosphere		
Dose Level	MMAD	GSD
2.07 mg/L	2.06 µm	2.05 µm

Chamber Environment	
Chamber Volume	100 liters
Airflow	28.3 LPM
Temperature	18 – 25° C
Relative Humidity	48-58%

Clinical Observations: Wet eyes, wet muzzle, unkempt appearance; fur coated with test article; licking of inside of mouth; wet anogenital area.

Gross Necropsy Findings: The lab reported that the test animals appeared normal and there were no gross necropsy findings.

DATA REVIEW FOR PRIMARY EYE IRRITATION TESTING (§81-4, 870.2400)

Product Manager: 33 **Reviewer:** I. Blackwell
MRID No.: 49467429 **Study Completion Date:** 6/24/2014
MB Research Project No.: MB 14-22490.04

Testing Laboratory: MB Research Laboratories
Author(s): Debra A. Hall, LATG

Quality Assurance (40 CFR §160.12): Included

Test Material: KX-6228, 2% (v/v) use-solution; "clear colorless liquid"
Dosage: 0.1 mL

Species: New Zealand White rabbit **Sex:** 1 male + 2 female
Weight: 3.2 – 3.4 kg **Age:** Approx. 24 weeks
Source: Covance Research Products, Inc.

Summary:

1. **Toxicity Category:** III

2. **Classification:** Acceptable

Procedure (Deviations From §81-4): None

Results:

Observations	(number "positive"/number tested)							
	Hour	Days						
	1	1	2	3	4	7	14	21
Corneal Opacity	0/3	0/3	0/3	0/3	---	0/3	---	---
Iritis	0/3	0/3	0/3	0/3	---	0/3	---	---
Conjunctivae								
Redness	0/3	1/3	2/3	0/3	---	0/3	---	---
Chemosis	0/3	0/3	0/3	0/3	---	0/3	---	---
Discharge	0/3	1/3	0/3	0/3	---	0/3	---	---

- - - = no observations at this point

DATA REVIEW FOR SKIN IRRITATION TESTING (§81-5, 870.2500)

Product Manager: 33 **Reviewer:** I. Blackwell
MRID No.: 49467428 **Study Completion Date:** 6/24/2014
MB Research Project No.:

Testing Laboratory: MB Research Laboratories
Study Director: Blair Yasso, B.S.

Quality Assurance (40 CFR §160.12): Included

Test Material: KX-6228, 2% (v/v) use-solution; "clear colorless liquid"
Dosage: 0.5 mL

Species: New Zealand White rabbit
Weight: 2.9 – 3.4 kg **Age:** Approx. 23 weeks
Source: Covance Research Products, Inc.

Summary:

1. **Toxicity Category:** IV

2. **Classification:** Acceptable

Procedure (Deviations From §81-5): None

Results: One hour after the administration of the test material, 1/3 test material-treated animals had very slight erythema. Twenty-four hours after application, 2/3 had very slight erythema and 1/3 very slight edema. Forty-eight hours after application, 2/3 had very slight erythema, 1/3 very slight edema and 1/3 flaking skin. Seventy-two hours after application 2/3 test material-treated animals had very slight erythema and 1/3 flaking skin. On Day 7 of the study, no erythema or edema was reported, 1/3 had flaking skin.

Special Comments: None

DATA REVIEW FOR DERMAL SENSITIZATION TESTING (§81-6, 870.2600)

Product Manager: 33 **Reviewer:** I. Blackwell
MRID No.: 49467424 **Study Completion Date:** 10/2/2014
Lab Study No.: MB 14-22489.26

Testing Laboratory: MB Research Laboratories
Author: Michael Carathers, B.S.

Quality Assurance (40 CFR §160.12): Included

Test Material: KX-6228 Concentrate; Clear colorless liquid, Lot P120331
Positive Control Material: alpha-Hexylcinnamaldehyde, 85%. 1-Chloro-2,4-Dinitrolbenzene in DMF (0.1% DNCB)

Species: CBA/J mice
Weight: 18.3 – 23.4 g **Age:** 9 weeks
Source: Jackson Laboratories

Method: Local Lymph Node Assay, BrdU ELISA

Summary:

1. **This Product is / is not a dermal sensitizer.**

2. **Classification:** Unacceptable

Procedure (Deviation From §81-6): The Office of Pesticide Programs has yet to accept the Local Lymph Node Assay, BrdU ELISA in support of acute toxicity testing.

Procedure:

Results:

Local Lymph Node Assay, Part 1				
Animal Group	Test Substance Concentration	Number of Mice in Group	Mean Stimulation Index	Test/ Vehicle Control Ratio
Vehicle Control	N/A		1.00	
Positive Control	25% HCA		1.72	
Positive Control	0.1% DNCB		1.85	
Test Group I	0.125%		0.67	
Test Group II	0.25%		1.23	
Test Group III	0.5%		1.18	
Test Group IV	1%		1.59	
Test Group V	2%		1.47	

Local Lymph Node Assay, Part 2				
Animal Group	Test Substance Concentration	Number of Mice in Group	Mean Stimulation Index	Test/ Vehicle Control Ratio
Vehicle Control	N/A		1.00	
Positive Control	25% HCA		1.61	
Positive Control	0.1% DNCB		2.59	
Test Group I	4%		0.73	
Test Group II	8%		0.95	